Effect of VAL66MET Mutation in BDNF Protein Regulating the Levels of BDNF in Type II Diabetic Patients with Arthritis, Hypertension and Obesity

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ABSTRACT

Objective: As Brain-derived neurotrophic factor (BDNF) is involved in major depressive disorder and neurodegenerative diseases, decreased levels of brain-derived neurotrophic factor (BDNF) have been implicated in case of Alzheimer's disease and depression. These disorders are associated with type 2 diabetes, and animal models suggest that BDNF plays a role in insulin resistance; it could be an important issue whether BDNF concentration is measured in serum or in plasma. We therefore explored the levels of BDNF in plasma of type 2 diabetic patients with Hypertension, Arthritis and Obesity. The molecular weight determination followed by sequence analysis going to explore the role of change in amino acid at 66th position which is responsible in regulation of BDNF level which further increase or decrease with insulin or glucose level in type 2 diabetics.

Key words: BDNF, ELISA, Mass spectrometry, Plasma, Protein Sequencing, Protein analysis, SDS-PAGE

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a secretory protein which is encoded by BDNF gene, belongs to the neurotrophin family of growth factor and exerts its actions by activating the tropomyosin-related kinase receptor B (TrkB). The molecular weight of BDNF is 27.8kDa, consisting of 247 amino acids & in humans it is present on chromosome 11, band p13.

.It is expressed in retina, CNS, motor neurons, kidneys, Prostate & in brain. It is active in Hippocampus, cortex & Basal forebrain. BDNF is involved in learning and memory formation and reduced BDNF levels in various brain regions have been implicated in the pathogenesis of neurodegenerative and psychiatric disorders. Recently it has become apparent that BDNF is present outside of the central nervous system (CNS) and circulates systemically. Studies using animal models have shown that conditions linked to metabolic and cardiovascular dysfunction, e.g. obesity, diabetes, heart disease, can be modified by manipulation of BDNF in the brain and in the peripheral circulation. In rodents, it has been suggested that BDNF can cross the Blood-brain barrier and one study indicated that cortical levels of BDNF correlate with platelet BDNF concentration. However, a recent study showed that BDNF concentration in the plasma is unrelated to levels found in the cortex and hippocampus. In spite of evidences from animal studies showing effects of BDNF on energy regulation and the cardiovascular system, little is known about BDNF plasma levels in human health including diabeties-2 as well as effect of change in amino acid and its correlation with BDNF level. In this study, we address this important issue, by SDS PAGE molecular weight determination followed by sequence analysis and BDNF level estimation by ELISA in diabetic patients aged between 40-65 and attempt to identify physiological and pathological parameters that may be correlated with plasma BDNF levels.

EXPERIMENTAL SECTION

Sample collection

Diabetic type-2 blood samples were collected in K2 EDTA vials from 20-25 different patients aged 40-65 with detailed case studies. Four of the patients were Diabetic as well as suffered from Hypertension, five were suffered from Arthritis and two were obese. We excluded the patients reported with severe viral infections.

MATERIALS AND METHODS

PLASMA EXTRACTION: 1ml of the blood sample was transferred into a fresh eppendorf tube under the aseptic conditions. Then, it was centrifuged at 8000 g for 10 minutes. A clear solution (plasma) was collected from the upper supernatant layer and preserved at -20^{o}C for further analysis.

SDS PAGE *(Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis)*

BDNF protein Identification was performed by Molecular weight analysis by using SDS page sample was run against the protein Marker (14.03Kd - 97.4Kd) and was subjected to electrophoresis in the presence of a detergent sodium dodecyl sulfate (SDS) and a reducing agent mercaptoethanol (b ME).

Separating Gel: 12% (30ml): 30% Acrylamide Stock- 12.0ml, 10% SDS-0.30ml, 1.5M Tris HCL (pH 8.8) - 7.50ml, 10% Ammonium Per Sulphate- 0.30ml, TEMED (added at last)- 0.012ml, Distilled water- 9.90ml.

Stacking Gel: 5% (10ml): 30% Acryl amide Stock-0.83ml, 10% SDS-0.05ml, 0.5M Tris HCL (pH 6.8) - 0.63ml, 10% Ammonium Per Sulphate-0.05ml, TEMED (added at last) - 0.005ml, Distilled water-4.0ml.

Sample Buffer: (10ml): Bromophenol – 0.8%, 2-mercaptoethanol-0.6ml, SDS-180mg, Glycerol-1ml, Stacking gel buffer-1.40ml, makeup the volume to 10ml.

Staining Solution (100 ml): Coomassie Brilliant Blue(R-250) - 200 mg, Ethanol- 50 ml, Acetic acid- 7 ml, Distilled water- 43ml.

De-staining Solution (100ml): Ethanol – 30ml, acetic acid-7ml, distilled water- 63ml.

The gel plates were assembled and tightly fitted in gel caster. 12% SDS resolving gel was prepared (APS and TEMED were added to the rest of the solution just before pouring) and poured into glass sandwich up to the mark with the resolving gel solution. To make the gel surface straight after polymerization, acryl amide solution was overlaid with water and removed by using filter paper and was allowed to polymerize. 5 % stacking solution was prepared and poured on the top of resolving gel. Then, the comb was inserted for polymerization.

Prior to loading, 10 µl of plasma was mixed with 10 µl of sample buffer and heat treated at 62^{o}C for 5 minutes. From this 10 µl of sample was taken, mixed with 10 µl of bromo phenol blue and loaded into the wells along with standard protein marker. It was electrophorised at constant voltage of 100-120 volts until the dye front reaches the top of the resolving gel. The gel was transferred to tray containing staining solution (5 volume of CBB) for at least one hour at room temperature, than gel was transferred into de staining solution until the bands are clearly visible. Then it was stored in7% acetic acid.

ESTIMATION OF BDNF LEVEL BY ELISA

BDNF levels were estimated by using the (Promega BDNF E_{max} ImmunoAssay System).designed for the sensitive and specific detection of BDNF in an antibody sandwich format and the readings were taken (Biorad 3550 micro reader).

PROTIEN SEQUENCING BY N-TERMINAL

Sequencing of proteins separated by SDS PAGE was performed by MALDI-TOF using matrix-assisted laser desorption ionization mass spectrophotometry (MALDI MS).

SEQUENCE ANALYSIS BY P-BLAST

Sequence analysis of BDNF sequence which we got from MALDI TOF was done by using protein BLAST tool from NCBI.

ANALYSIS FOR SNP DETECTION

By using various Insilco tools the single nucleotide polymorphism was identified and a mutation was detected at 66th position of the BDNF protein.

RESULT & DISSCUSSION

BDNF Protein was separated by SDS PAGE and visualized by Coommasie brilliant blue staining is depicted in fig.1.

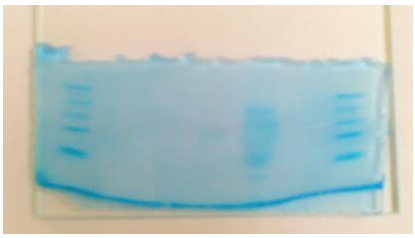

Figure 1: SDS PAGE-image showing Lane 1 Marker (14.7-97.8KD), Lane: 4-purified sample, Lane 5: heamolysed Sample, Lane 7: Marker

The BDNF sequence got after the MALDI TOF analysis:

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MTILFLTMVISYFGCMKAAPMKEANIRGQGGLAYP
GVRTHGTLESVNGPKAGSRGLTSLADTFEHMIEEL
LDEDQKVRPNEENNKDADLYTSRVMLSSQVPLEPP
LLFLLEEYKNYLDAANMSMRVRRHSDPARRGELSV
CDSISEWVTAADKKTAVDMSGGTVTVLEKVPVSKG
QLKQYFYETKCNPMGYTKEGCRGIDKRHWNSQCRT
TQSYVRALTMDSKKRIGWRFIRIDTSCVCTLTIKR
GR

The BDNF levels were measured by ELISA

Table 1: Table showing BDNF levels in different patients

Name of patient	BDNF Levels	OTHER COMPLICATIONS OR DISEASES
Patient 1	750.39	-
Patient 2	925.78	-
Patient 3	842.78	Hypertension
Patient 4	914.35	Arthritis
Patient 5	735.23	Arthritis
Patient 6	711.24	-
Patient 7	815.73	-
Patient 8	632.46	Arthritis
Patient 9	838.72	-

Patient 10	785.14	-
Patient 11	988.13	Hypertension
Patient 12	695.83	-
Patient 13	810.05	-
Patient 14	912.35	Gastric
Patient 15	818.29	-
Patient 16	937.24	Obesity
Patient 17	852.12	Arthritis & Obesity
Patient 18	961.35	Dry skin
Patient 19	878.13	-
Patient 20	796.66	Hypertension
Patient 21	906.72	Hypertension,Breathing problem
Patient 22	909.42	Arthritis
Patient 23	885.27	-

Sequence analysis performed by p-blast showed 100% similarity and 0% E-Value with .gb|AAA96140.1| gb|AAH29795.1| gb|AAT74399.1| gb|AAX42591.1| gb|ABM81737.1| gb|ABM84892.1|

The SNP analysis detection reported the change of Val to Met at 66th position. The above study shows that the BDNF levels are independent with Type II Diabetes (by ELISA).The mutation reported at the 66th amino acid (Val/Met) doesn't show any effect on the levels of the BDNF in Type II Diabetes and patients with other complications like Arthritis, Obesity and Hypertension.

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